Fish & Shellfish Immunology 47 (2015) 706–711

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry

Seyed Hossein Hoseinifar [a, *], Mohsen Khalili [b], Rudabeh Rufchaei [c], Mojtaba Raeisi [d, e], Marzieh Attar [b], Héctor Cordero [f], M. Ángeles Esteban [f]

[a] Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[b] Medical Cellular & Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[c] Inland Water Aquaculture Institute, Specialized Research Station of Aquatics Nutrition and Live Food, BandareAnzali, Iran
[d] Cereal Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[e] Department of Public Health, School of Health, Golestan University of Medical Sciences, Gorgan, Iran
[f] Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, 30100, Murcia, Spain

ARTICLE INFO

Article history:
Received 2 August 2015
Received in revised form
16 September 2015
Accepted 29 September 2015
Available online 9 October 2015

Keywords:
Date palm extract
Skin mucosal immunity
Gene expression
Growth
Common carp (*Cyprinus carpio*)

ABSTRACT

The aim of this study was to investigate the effects of date palm fruit extracts (DPFE) on skin mucosal immunity, immune related genes expression and growth performance of fry common carp (*Cyprinus carpio*). One hundred and twenty specimens (4.06 ± 0.13 g) were supplied and allocated into six aquaria; specimens in three aquaria were fed non-supplemented diet (control) while the fish in the other 3 aquaria were fed with DPFE at 200 ml kg^{-1}. At the end of feeding trial (8 weeks) skin mucus immune parameters (total immunoglobulins, lysozyme, protease and alkaline phosphatase activity) and immune related gene expression (tumor necrosis factor α [*tnfa*], lysozyme [*ly*] and interleukin-1-beta, [*il1b*]) in the head-kidney were studied. The results revealed that feeding carp fry with 200 ml kg^{-1} DPFE remarkably elevated the three skin mucus immune parameters tested ($P < 0.05$). However, evaluation of immune related gene expression demonstrated that the expression of *tnfa* and *il1b* was considerably decreased ($P < 0.05$) in fish fed DPFE diet, while the expression of *ly* remained similar ($P > 0.05$) compared to control fish (fed control diet). Furthermore, growth performance parameters were significantly improved in fry fed DPFE ($P < 0.05$). More studies are needed to understand different aspects of DPFE administration in fry mucosal immunity.

1. Introduction

Global fish production continues increasing, and aquaculture is one of the fastest-growing food producing sectors [1]. Due to the continuous augment of the aquaculture production, there is a great interest in improving fish diets from reared fish. At present, satisfactory nutrition is considered essential for fish, not only to avoid deficiency signs but also to maintain adequate animal well-being and performance. Furthermore, there is much evidence that normal diets enriched with specific nutrients (such as vitamins, proteins, amino acids, essential fatty acids or minerals) may improve the health condition of the fish and the disease resistance (reviewed by Ref. [2]). In most of the occasions, these supplements have been tested for their antioxidant properties [3,4]. However, many other functional constituents (mainly probiotics, prebiotics and immunostimulants) have also been studied due to their capacity for increasing growth and/or feed efficiency, health status [5,6], fish immune activity [7–10], stress tolerance [11,12] or disease resistance [13–17].

In the past two decades, substantial progress has been made about our knowledge regarding bioactive components in plant foods and their direct links to human health [18]. In fact, medicinal foods have recently received immense interest among the health professionals and public. Consequently, the global health market has been swamped with such products claiming to improve health as well as prevent chronic diseases. Among the fruits, one of the most popular is the date palm fruits (DPF). DP (*Phoenix dactylifera*

* Corresponding author.
E-mail address: hoseinifar@gau.ac.ir (S.H. Hoseinifar).

http://dx.doi.org/10.1016/j.fsi.2015.09.046
1050-4648/

L.) is an important, and one of the oldest trees (5500–3000 BC) cultivated by man and is closely tied to the history of human civilization [19,20]. However, only recently, the health benefits of dates have been demonstrated after *in vitro* and animal studies; besides that, the identification and quantification of several phytochemical presents on them have been pursued worldwide [21,22]. Preclinical studies have shown that the DPF possesses numerous and important functions in humans, among them free radical scavenging, antioxidant, immunostimulant, antimutagenic, antimicrobial, anti-inflammatory, anti-cancer and gastro-, hepato- and nephro-protective [23]. To the best of our knowledge, our team was the first one focuses on the effects of dietary administration of DPFE on fish mucosal immune response. In our previous study we demonstrate the antioxidant effects of dietary supplementation of probiotics and DPFE in the mucose of gilthead seabream (*Sparus aurata* L.). The mucosal surfaces of fish act as the first line of defense against the pathogens that can be present in the aquatic environment. However, the immune repertoire functioning at these interfaces is still poorly understood. Taking into account these previous data, the present study has been undertaken to know the effects of DPFE on skin mucosal immunity, immune related genes expression as well as growth performance of fry common carp (*Cyprinus carpio*).

2. Material and methods

2.1. Fish culture and feeding trial

One hundred and twenty common carp fry (4.06 ± 0.13 g) were obtained from a private sector fish farm and transferred to the Aquaculture Laboratory of Gorgan University of Agricultural Sciences and Natural Resources (Iran). Fish were distributed in six aquaria (100 L) at a density of 20 fish per aquaria and acclimatized for two weeks prior to experiment. The water quality parameters including temperature, dissolved oxygen, pH were monitored daily and maintained at 26.54 ± 1.29 °C, 7.03 ± 0.13 mg L^{-1}, 7.21 ± 0.28, respectively. Common carp fry were hand-fed to apparent satiation twice a day (09:00 h and 15:00 h) for eight weeks. Utmost care was taken to avoid feed losses. Treatments were investigated under static aerated water conditions with a 50% water change every day.

2.2. Preparation of DPFE extracts and experimental diet

Palm fruit extracts of the Tunisian Degla variety were prepared as previously described [24]. The experimental diets were prepared by supplementing the basal diet (Table 1) without (0 ml kg^{-1}, control diet) or with 200 ml kg^{-1}. The ingredients were blended thoroughly in a mixer and pelleted using a meat grinder equipped with a 2-mm die. The pelleted diets were air-dried and stored in plastic bag at 4 °C until further use [25].

2.3. Skin mucosal immune response

2.3.1. Mucus collection

At the end of the feeding trial, twelve 24 h-starved fish were randomly selected from each treatment, anesthetized with clove oil (5 mg L^{-1}) and then transferred into polyethylene bags containing 10 ml of 50 mM NaCl [26]. The bags were gently shaken for approximately 1 min to release epidermal mucus. Thereafter, the collected mucus samples were immediately transferred to 15 ml sterile centrifuge tubes and centrifuged (5810R Eppendorf, Engelsdorf, Germany) (1500 × g, 10 min, 4 °C). The supernatant was stored at −80 °C for future analysis.

Table 1
Dietary formulations (%) and proximate composition.

Ingredient	Control
Fish meal	40.0
Wheat flour	21.0
Soybean meal	13.5
Gluten	5.5
Soybean oil	6.0
Fish oil	6.0
Mineral premix[a]	3.0
Vitamin premix[a]	2.0
Binder[b]	2.0
Anti fungi[c]	0.5
Antioxidant[d]	0.5
Proximate analysis (% dry matter basis)	
Dry matter	89.50
Crude protein	38.22
Crude lipid	10.24
Ash	3.45
Fiber	11.20
NFE[e]	26.39
Energy (MJ kg^{-1})[f]	17.55

[a] Premix detailed by (Hoseinifar et al., 2014).
[b] Amet binder™, Mehr Taban-e− Yazd, Iran.
[c] ToxiBan antifungal (Vet-A-Mix, Shenan-doah, IA).
[d] Butylated hydroxytoluene (BHT) (Merck, Germany).
[e] Nitrogen-free extracts (NFE) = dry matter − (crude protein + crude lipid + ash + fiber).
[f] Gross energy (MJ kg^{-1}) calculated according to 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.0 kJ g^{-1} for NFE.

2.3.2. Total immunoglobulin

Siwicki and Anderson [27] method was used for determination of skin mucus total immunoglobulin (Ig) levels. Briefly, mucus total protein content was measured using a micro protein determination method (C-690; Sigma). Thereafter, the immunoglobulin molecules precipitated down using a 12% solution of polyethylene glycol (Sigma). The difference in protein contents prior and after immunoglobulin molecules precipitation is considered as the Ig content.

2.3.3. Lysozyme activity

Lysozyme activity of common carp fry skin mucus fed experimental diets was determined using a turbidimetric method based on the lysis of the lysozyme-sensitive Gram-positive bacterium *Micrococcus lysodeikticus* (Sigma) [28]. A unit of lysozyme activity was defined as the amount of samples causing a decrease in absorbance of 0.001 min^{-1}.

2.3.4. Protease activity

The skin mucus protease activity was measured according to Palaksha et al. [29] using the azocasein hydrolysis assay. The enzymatic activities were expressed as specific activities (U mg $protein^{-1}$). The mucus protein level was determined by the Bradford [30] method.

2.3.5. Alkaline phosphatase activity

The activity alkaline phosphatase (ALP) in the mucus was measured using a commercial kit (Pars Azmoun Co., Iran). Samples were prepared according to the manufacturer protocol, and the absorbance was read at 405 nm (Smith et al., 2000).

2.4. Immune related genes expression studies

Relative gene expression was analyzed in head kidney tissue from nine fish per treatment using Real-Time PCR [24]. Head kidney samples from the same group of fish were pooled and placed into sterile and DNAase/RNAase free tubes. According to Sinagene/Iran

corporation kit, the total RNA was extracted with RNXplus. The quantity and quality of extracted RNA sample were examined and verified with spectrophotometry, in 260 and 280 nm wave lengths.

The RNA was then treated with DNase I (Fermentas) to remove genomic DNA contamination. Complementary DNA (cDNA) was synthesized from 5 μg of total RNA using the SuperScript III reverse transcriptase (Invitrogen) with an oligo-dT18 primer. The expression of fifteen selected genes was analyzed by real-time PCR, which was performed with an ABI PRISM 7500 instrument (Applied Biosystems) using SYBR Green PCR Core Reagents (Applied Biosystems). Reaction mixtures [containing 10 μl of 2× SYBR Green supermix, 5 μl of primers (0.6 μM each) and 5 μl of cDNA template] were incubated for 10 min at 65 °C, followed by 40 cycles of 15 s at 95 °C, 30 min at 55 °C, and finally 5 min at 85 °C. For each mRNA, gene expression was corrected by the beta-actin content in each sample. The primers used are shown in Table 2. In all cases, each PCR was performed with triplicate samples.

2.5. Growth performance

The effects of dietary administration of DPFE for 8 weeks on growth performance parameters as well as survival rates were calculated using the following formula:

$$\text{Weight gain} = W_2(g) - W_1(g);\ \text{Specific growth rate (SGR)} = 100(\ln W_2 - \ln W_1)/T;$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)}/\text{weight gain (g)};$$

$$\text{Survival rate} = (N_f/N_i) \times 100;$$

Where W_1 is the initial weight, W_2 is the final weight; T is the number of days in the feeding period, N_i is the initial number of fish, and N_f is the final number of fish.

2.6. Statistical analysis

Data of gene in figure are expressed as fold increase (mean ± standard error, SE), obtained by dividing each sample value by the mean control value at the same sampling time. Values higher than 1 express an increase while values lower than 1 express a decrease in the indicated gene. Data were statistically analyzed by the t-Student test using SPSS software v19 (SPSS, USA) to determine differences between control and experimental diet group. Asterisks denote statistically significant differences when $P < 0.05$.

3. Results

Diet supplemented with DPFE has marked positive effects on carp fry skin mucus immune parameters (Figs. 1–4). Evaluation of total Ig levels in skin mucus revealed a significant increase in DPFEs fed fish compared to control group ($P < 0.05$) (Fig. 1). Similarly, remarkable elevations were noticed in skin mucus lysozyme and protease activity of common carp fry fed 200 ml kg^{-1} DPFE ($P < 0.05$) (Figs. 2 and 3). Furthermore, compared to control group, carp fry fed 200 ml kg^{-1} DPFE also showed significantly higher skin mucus ALP activity ($P < 0.05$).

Supplementation of fish diet with DPFEs caused changes in the expression of the genes analyzed (see Table 2) on head-kidney of common carp fry compared with the control group. The expression of the genes encoding *tnfa* and *il1b* was significantly decreased in fish fed enriched diet compared to the values find on head kidney from control fish (fed control diet) ($P < 0.05$) (Fig. 5). On the other hand, regarding gene encoding *ly*, a non-significant increase was observed in head kidney from fish fed enriched diet, compare to the control fish (Fig. 5).

The effects of dietary DPFEs on growth performance parameters, feed utilization and survival rate of common carp fry are presented in Table 3. The results revealed significant difference between final weight, weight gain, SGR and FCR of fry fed control or DPFE supplemented diets ($P < 0.05$). Administration of 200 ml kg^{-1} DPFE in carp fry diet remarkably improved growth performance and feed utilization. Survival rates were 100% in all treatments and no mortality occurred during the feeding trial.

Table 2
Primers sequences for the study of selected immune related genes expression in common carp fry.

Genes	Accession number	Primer sequence
Lysozyme-C	AB027305	F: 5′ GTGTCTGATGTGGCTGTGCT 3′ R: 5′ TTCCCCAGGTATCCCATGAT 3′
TNF-α	AJ311800	F: 5′ GGTGATGGTGTCGAGGAGGAA 3′ R: 5′ TGGAAAGACACCTGGCTGTA 3′
IL-1β	AB010701	F: 5′ ACCAGCTGGATTTGTCAGAAG 3′ R: 5′ ACATACTGAATTGAACTTTG 3′
β actin	M24113	F: 5′ AGACATCAGGGTGTCATGGTTGGT 3′ R: 5′ CTCAAACATGATCTGTGTCAT 3′

4. Discussion

Modulation of fish immune response using probiotic, prebiotics and herbal immunostimulant received increasing interest during the past decades [7,9,15,31–34]. The beneficial results obtained in various studies, encourage further researches on potential immune modulatory nature of different types of environment-friendly dietary supplements [10,31]. Recently, the results of *in vitro* and animal studies revealed health benefits of the DPF (*P. dactylifera*) [20,21,24]. However, there is no available information on the effects of dietary DPFE on fish mucosal immune response and growth performance.

DPFs are a rich source of a wide variety of non-nutritive, nutritive, and bioactive compounds, including flavonoids, phenolics, anthocyanins and phenolic acids, as well as nutritive compounds such as sugars, essential oils, vitamins, and minerals. Bioactive compounds from DPF have potent antioxidant,

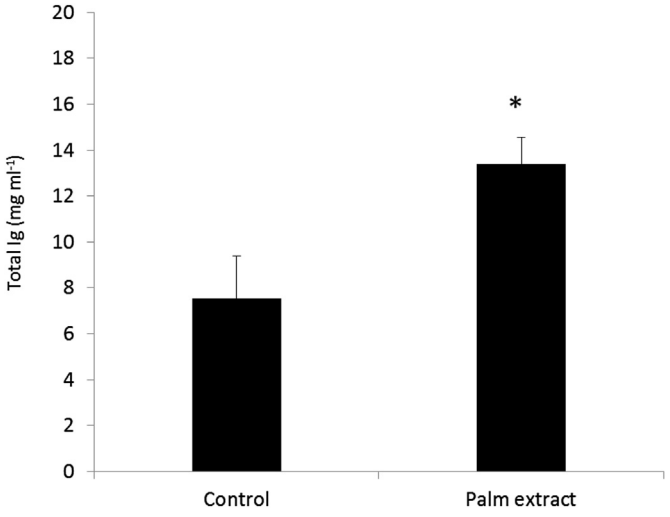

Fig. 1. The skin mucus total immunoglobulin level in common carp fry fed experimental diets fed two levels of date palm fruit extract (0 and 200 ml kg^{-1}) for 8 weeks. Bar assigned with asterisk denotes significant difference ($P < 0.05$); Values are presented as the mean ± SE.

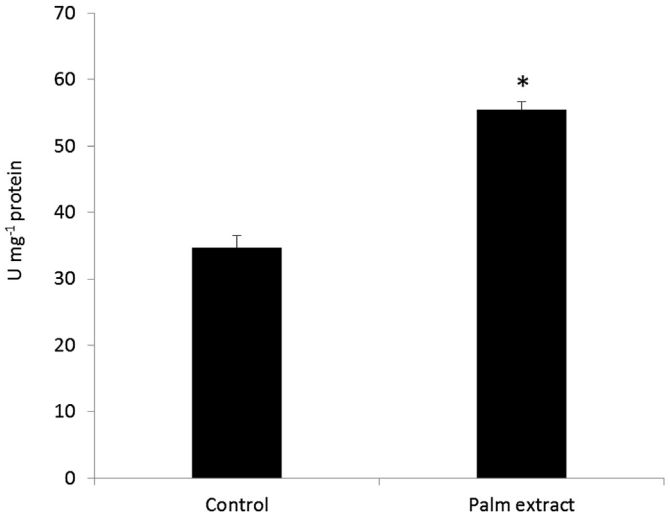

Fig. 2. The skin mucus lysozyme activity in common carp fry fed experimental diets fed two levels of date palm fruit (0 and 200 ml kg^{-1}) for 8 weeks. Bar assigned with asterisk denotes significant difference ($P < 0.05$); Values are presented as the mean ± SE.

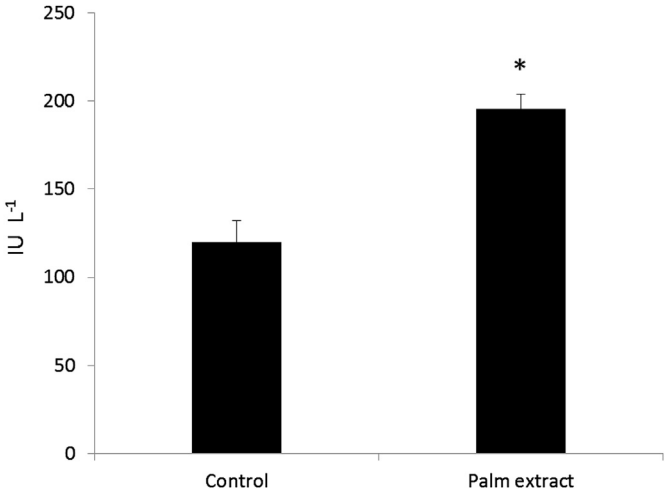

Fig. 4. The skin mucus Alkaline phosphatase activity in common carp fry fed experimental diets fed two levels of date palm fruit (0 and 200 ml kg^{-1}) for 8 weeks. Bar assigned with asterisk denotes significant difference ($P < 0.05$); Values are presented as the mean ± SE.

anticancer, antimutagenic, antimicrobial, anti-inflammatory, and antineurodegenerative properties, both *in vitro* and *in vivo* [21,22]; although those activities have not been demonstrated in fish. Analysis of DPF composition has revealed that they contained many components that can be involved in the demonstrated nutritional and health beneficial properties. Among these phytonutrients carotenoids (alpha-, beta- and gamma-carotenes), vitamin E (tocopherols and tocotrienols), sterols (sitosterol, stigmasterol and campesterol), phospholipids, glycolipids and squalene are included. In addition, it has been recently reported different water-soluble powerful antioxidants, such as phenolic acids and flavonoids. Owing to its high content of phytonutrients with antioxidant properties, the possibility exists that DPF offers some health advantages by reducing lipid oxidation, oxidative stress and free radical damage. Accordingly, use of DPF or its phytonutrient-rich fractions, particularly water-soluble antioxidants, may confer some protection against a number of disorders or diseases [35]. To our knowledge, this is the first attempt to investigate the effects of DPFE on mucosal immune response, immune related genes expression as well as growth performance of common carp fry.

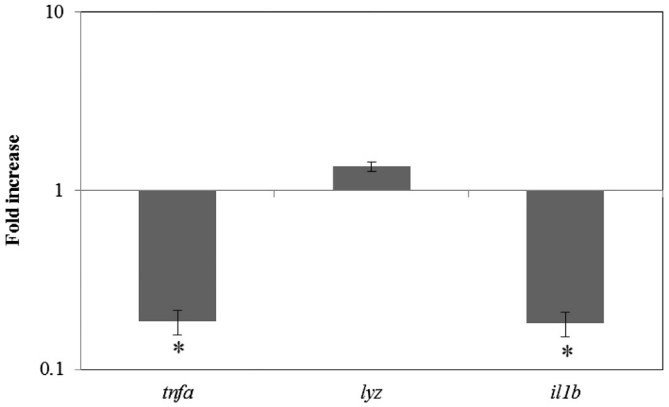

Fig. 5. Expression of three immune-relevant genes, tumor necrosis factor-alpha (*tnfa*) interleukin-1-beta (*il1b*) and lysozyme (*lys*) determined by real-time PCR in HK of common carp fed two levels of date palm fruit (0 and 200 ml kg^{-1}) for 8 weeks. The bars represent the mean ± S.E (n = 9) fold increase relative to control. Asterisks denote significant differences between control and treated groups ($P < 0.05$).

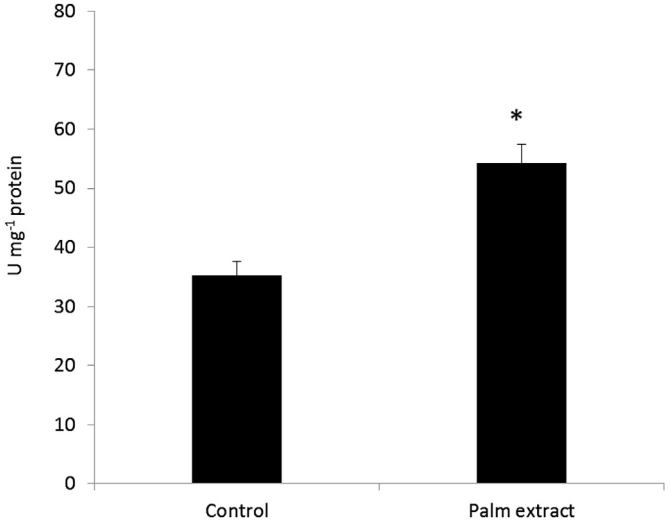

Fig. 3. The skin mucus protease activity in common carp fry fed experimental diets fed two levels of date palm fruit (0 and 200 ml kg^{-1}) for 8 weeks. Bar assigned with asterisk denotes significant difference ($P < 0.05$); Values are presented as the mean ± SE.

Table 3
Growth performance and feed utilisation of common carp fry fed experimental diets contain two levels of date palm fruit (0 and 200 ml kg^{-1}) for 8 weeks. Values are presented as the mean ± SE.

	Palm extract supplemented diet	
	0	200 ml kg^{-1}
Initial length (cm)	6.70 ± 0.25^a	6.51 ± 0.14^a
Initial weight (g)	4.12 ± 0.13^a	4.01 ± 0.16^a
Final length (cm)	9.42 ± 0.11^a	10.25 ± 0.07^b
Final weight (g)	9.79 ± 0.28^a	13.18 ± 0.33^b
WG (g)	5.67 ± 0.28^a	9.17 ± 0.49^b
SGR	1.76 ± 0.11^a	2.42 ± 0.13^b
FCR	2.68 ± 0.11^a	2.21 ± 0.13^b
Survival (%)	100^a	100^a

Values in a row with different superscripts denote a significant difference ($P < 0.05$).

Common carp fry has been selected for the present study because it is considered to be a very important aquaculture species in many Asians and some European countries [36]. Furthermore, in this study we have used the same DPF variety and extracts concentrations that tested in our previous work [24] because, we demonstrated significant antioxidant properties in gilthead seabream skin mucosa.

The overall results of the present study revealed elevated mucosal immune parameters include total Ig, lysozyme, protease and ALP activity in common carp fry fed DPFE supplemented diet. Although, there is no report on the effects of dietary DPFE on fish immune response, similar to the results of the present study, Karasawa et al. [37] reported that hot water extract from matured fruit of the date palm tree (*P. dactylifera* L.) stimulates the cellular immune system in mice. Furthermore, a study on Artemia revealed that the use of DPFE has remarkably improved culture conditions as well as inhibiting bacterial pathogens under *in vivo* conditions [38]. Furthermore, administration of DPFE in gilthead seabream (*S. aurata* L.) elevated the expression of the antioxidant enzyme genes (superoxide dismutase, catalase and glutathione reductase) in the fish mucosa (including gut, skin and gill) [24]. Considering the protective effect of fish skin mucus as the first defense line against pathogens, elevation of mucosal immune response obtained through supplementation of diet with DPFE is of high interests, especially in early stages of life. However, determinations of the mode of action of DPFE on fish mucosal immune response merit further researches.

In the present work, real time PCR was performed to analyze the expression of three genes with a key role in the fish immune response. These genes were selected based on different criteria. Briefly, although originally identified by its ability to kill certain tumor cells *in vivo* [39], TNF-alpha is one of the best studied fish cytokine with multiple biological effects. It regulates the immune responses and it is involved in systemic inflammation and regulation of immune cells, besides this it mediates cell death and survival [40]. It is produced chiefly by activated macrophages as a membrane or secreted form [41]. TNF-alpha is a mediator of the antimicrobial defense mechanisms and it is able to eliminate different pathogens by inducing a variety of cellular responses (e.g. chemotaxis and phagocytosis); due to all these properties it is considered an excellent biomarker and health indicator for both mammals and fish [42]. Interleukin-1-beta is another major mediator of inflammation and can induce the expression of a wide variety of non-structural, function-associated genes during infection [43]. It plays a key role in the host response to microbial invasion and tissue injury [44] due its ability to enhance phagocyte activity, macrophage proliferation, lysozyme synthesis and leukocyte migration [45]. Finally, lysozyme is a bactericidal enzyme present in serum, mucus and lymphoid tissues of most fish species [46], being an important part of the humoral innate immune system. These three genes were selected to test the effect of the assayed supplement diets on gene expression in the head-kidney (the main hematopoietic organ in fish). The present results revealed that the expression of each selected gene was differentially affected as a consequence of the dietary administration of DPFE. In fact, while the expression of lysozyme remained similar compared to control fish (fed control diet), the expression of genes encoding *tnfa* and *il1b* was significantly decreased in fish fed DPFE supplemented diet. Future studies are needed to understand the effect of DPFE on immune system activities and gene expression.

Evaluation of growth performance of common carp fry following eight weeks feeding on date palm extracts supplemented diet revealed improved growth performance parameters as well as diet utilization. Although, there is no data available on the effects of date palm extracts, the growth enhancement can be attributed to better nutritional status offered by dietary date palm extracts. It has been proved that date palm has high nutritional value due to its fiber content, vitamins (A, C, B1 and B2), enzymes (phytase, invertase and peroxidase) as well as essential minerals (calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese, among others) [24].

To conclude, the present results demonstrate that dietary DPFE improves the natural defenses present in skin mucus of fry common carp. To the best of our knowledge, this is the first work studying the effects of this food additive on the skin mucosal immunity as well as on the expression of different genes in fish head-kidney. DPFE seems to be good natural antioxidants [24] and immunostimulants for fish, and they could potentially be considered as a functional food ingredient for farmed fish as a preventive action for protection against free radicals stressors and/or microorganism induced alterations or disorders. Furthermore, the positive effect of such extracts on fry growth seems to indicate that dietary DPFE has many more beneficial properties in the fish, which deserves future studies.

Acknowledgments

The present study is partially funded by Gorgan University of Agricultural Sciences and Natural Resources (Grant Number: 93-323-6). We would like to thanks financial supports of Research Affairs. The funder had no role in the design, analysis or writing of this article. The authors would like to thanks the kind helps of the staff at Medical Cellular & Molecular Research Center, Talghani Children University hospital. H. Cordero wishes to thank the *Ministerio de Economía y Competitividad* for a F.P.I. fellowship. The financial support of the Spanish Ministry of Economy and Competitiveness under Grant no. AGL-2011-30381-C03-01 and of the *Fundación Séneca de la Región de Murcia* (Spain) (Grant no. 04538/GERM/06, *Grupo de Excelencia de la Región de Murcia*) is gratefully acknowledged.

References

[1] FAO. Aquaculture Department, The State of World Fisheries and Aquaculture 2014, Food and Agriculture Organization of the United Nations, Rome, 2014, p. 243.
[2] A. Oliva-Teles, Nutrition and health of aquaculture fish, J. Fish. Dis. 35 (2012) 83–108.
[3] X.-Y. Li, H.-H. Huang, K. Hu, Y. Liu, W.-D. Jiang, J. Jiang, et al., The effects of dietary thiamin on oxidative damage and antioxidant defence of juvenile fish, Fish. Physiol. Biochem. 40 (2014) 673–687.
[4] J. Zhao, Y. Liu, J. Jiang, P. Wu, W. Jiang, S. Li, et al., Effects of dietary isoleucine on the immune response, antioxidant status and gene expression in the head kidney of juvenile Jian carp (*Cyprinus carpio* var. Jian), Fish Shellfish Immunol. 35 (2013) 572–580.
[5] N.V. Hai, Research findings from the use of probiotics in tilapia aquaculture: a review, Fish Shellfish Immunol. 45 (2015) 592–597.
[6] J. Galina, G. Yin, L. Ardo, Z. Jeney, The use of immunostimulating herbs in fish. An overview of research, Fish Physiol. Biochem. 35 (2009) 669–676.
[7] B.C. De, D. Meena, B. Behera, P. Das, P.D. Mohapatra, A. Sharma, Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses, Fish Physiol. Biochem. 40 (2014) 921–971.
[8] C.M.A. Caipang, C.C. Lazado, 9-Nutritional impacts on fish mucosa: immunostimulants, pre- and probiotics, in: B.H.B. Peatman (Ed.), Mucosal Health in Aquaculture, Academic Press, San Diego, 2015, pp. 211–272.
[9] S.K. Song, B.R. Beck, D. Kim, J. Park, J. Kim, H.D. Kim, et al., Prebiotics as immunostimulants in aquaculture: a review, Fish Shellfish Immunol. 40 (2014) 40–48.
[10] S.H. Hoseinifar, M. Esteban, A. Cuesta, Y.-Z. Sun, Prebiotics and fish immune response: a review of current knowledge and future perspectives, Rev. Fish. Sci. Aquac. 23 (2015) 315–328.
[11] M.E. Yonar, Protective effect of lycopene on oxidative stress and antioxidant status in *Cyprinus carpio* during cypermethrin exposure, Env. Toxicol. 28 (2013) 609–616.
[12] B. Liu, P. Xu, J. Xie, X. Ge, S. Xia, C. Song, et al., Effects of emodin and vitamin E on the growth and crowding stress of Wuchang bream (*Megalobrama amblycephala*), Fish Shellfish Immunol. 40 (2014) 595–602.
[13] L. Verschuere, G. Rombaut, P. Sorgeloos, W. Verstraete, Probiotic bacteria as

biological control agents in aquaculture, Microbiol. Mol. Biol. Rev. 64 (2000) 655–671.
[14] C. Lim, C.D. Webster, C.-S. Lee, Feeding Practices and Fish Health. Dietary Nutrients, Additives, and Fish Health, John Wiley & Sons, Inc., 2015, pp. 333–346.
[15] C. Pohlenz, D.M. Gatlin III, Interrelationships between fish nutrition and health, Aquaculture 431 (2014) 111–117.
[16] V. Kiron, Fish immune system and its nutritional modulation for preventive health care, Anim. Feed Sci. Technol. 173 (2012) 111–133.
[17] B.T. Bich Hang, N.T. Phuong, P. Kestemont, Can immunostimulants efficiently replace antibiotic in striped catfish (*Pangasianodon hypophthalmus*) against bacterial infection by *Edwardsiella ictaluri*? Fish Shellfish Immunol. 40 (2014) 556–562.
[18] C. Abuajah, A. Ogbonna, C. Osuji, Functional components and medicinal properties of food: a review, J. Food Sci. Technol. 52 (2015) 2522–2529.
[19] W. Barreveld, Date Palm Products. Agricultural Services Bulletin No. 101, FAO, Rome, 1993.
[20] M. Chandrasekaran, A.H. Bahkali, Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology–review, Saudi J. Biol. Sci. 20 (2013) 105–120.
[21] N. Chaira, M.I. Smaali, M. Martinez-Tomé, A. Mrabet, M.A. Murcia, A. Ferchichi, Simple phenolic composition, flavonoid contents and antioxidant capacities in water-methanol extracts of Tunisian common date cultivars (*Phoenix dactylifera* L.), Int. J. Food Sci. Nutr. 60 (2009) 316–329.
[22] P.K. Vayalil, Date fruits (*Phoenix dactylifera* L.): an emerging medicinal food, Crit. Rev. Food Sci. Nutr. 52 (2012) 249–271.
[23] L.O. Dragsted, M. Strube, J.C. Larsen, Cancer-protective factors in fruits and vegetables: biochemical and biological background, Pharmacol. Toxicol. 72 (1993) 116–135.
[24] M. Esteban, H. Cordero, M. Martínez-Tomé, A. Jiménez-Monreal, A. Bakhrouf, A. Mahdhi, Effect of dietary supplementation of probiotics and palm fruits extracts on the antioxidant enzyme gene expression in the mucosae of gilthead seabream (*Sparus aurata* L.), Fish Shellfish Immunol. 39 (2014) 532–540.
[25] S.H. Hoseinifar, N. Soleimani, E. Ringø, Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (*Cyprinus carpio*) fry, Br. J. Nutr. 112 (2014) 1296–1302.
[26] S. Subramanian, S.L. MacKinnon, N.W. Ross, A comparative study on innate immune parameters in the epidermal mucus of various fish species, Comp. Biochem. Physiol. Biochem. Mol. Biol. 148 (2007) 256–263.
[27] A.K. Siwicki, D.P. Anderson, Nonspecific defense mechanisms assay in fish: II. potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum, in: Fish Diseases Diagnosis and Prevention Methods, 1993, pp. 105–112. Olsztyn, Poland.
[28] N.E. Demers, C.J. Bayne, The immediate effects of stress on hormones and plasma lysozyme in rainbow trout, Dev. Comp. Immunol. 21 (1997) 363–373.
[29] K. Palaksha, G.-W. Shin, Y.-R. Kim, T.-S. Jung, Evaluation of non-specific immune components from the skin mucus of olive flounder (*Paralichthys olivaceus*), Fish Shellfish Immunol. 24 (2008) 479–488.
[30] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[31] M. Reverter, N. Bontemps, D. Lecchini, B. Banaigs, P. Sasal, Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives, Aquaculture 433 (2014) 50–61.
[32] D.M. Gatlin, Prebiotics. Dietary Nutrients, Additives, and Fish Health, John Wiley & Sons, Inc., 2015, pp. 271–281.
[33] G. Jeney, L.D. Wet, Z. Jeney, G. Yin, Plant Extracts. Dietary Nutrients, Additives, and Fish Health, John Wiley & Sons, Inc., 2015, pp. 321–332.
[34] R. Harikrishnan, C. Balasundaram, M.-S. Heo, Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish, Aquaculture 317 (2011) 1–15.
[35] N. Wattanapenpaiboon, M.L. Wahlqvist, Phytonutrient deficiency: the place of palm fruit, Asia. Pac. J. Clin. Nutr. 12 (2003) 363–368.
[36] M.M. Rahman, Role of common carp (*Cyprinus carpio*) in aquaculture production systems, Front. Life Sci. (2015) 1–12, http://dx.doi.org/10.1080/21553769.2015.1045629.
[37] K. Karasawa, Y. Uzuhashi, M. Hirota, H. Otani, A matured fruit extract of date palm tree (*Phoenix dactylifera* L.) stimulates the cellular immune system in mice, J. Agric. Food Chem. 59 (2011) 11287–11293.
[38] A. Mahdhi, A. Bahi, D. Mzah, A. Bakhrouf, Use of mixture design to construct a consortium of date palm (*Phoenix dactylifera* L.) fruit extract and potentially probiotic bacillus strain to confer protection against vibriosis in Artemia culture, J. Sci. Food Agric. 93 (2013) 3850–3855.
[39] L. Old, Tumor necrosis factor (TNF), Science 230 (1985) 630–632.
[40] T. Krakauer, J. Vilcek, J.J. Oppenheim, Proinflammatory cytokines: TNF and IL-1 families, chemokines, TGF-b, and others, in: W. Paul (Ed.), Fundamental Immunology, fourth ed., Lippincott-Raven Publishers, Philadelphia, 1999, pp. 775–812.
[41] S. Hong, R. Li, Q. Xu, C.J. Secombes, T. Wang, Two types of TNF-α exist in teleost fish: phylogeny, expression, and bioactivity analysis of Type-II TNF-α3 in rainbow trout *Oncorhynchus mykiss*, J. Immunol. 191 (2013) 5959–5972.
[42] F.W. Goetz, J.V. Planas, S. MacKenzie, Tumor necrosis factors, Dev. Comp. Immunol. 28 (2004) 487–497.
[43] S. Bird, J. Zou, T. Wang, B. Munday, C. Cunningham, C.J. Secombes, Evolution of interleukin-1β, Cytokine Growth Factor Rev. 13 (2002) 483–502.
[44] C.A. Dinarello, Interleukin-1 family (IL-1F1, F2), in: A. Thomson, M. Lotze (Eds.), The Cytokine Handbook, Elsevier Science Ltd., London, 2003, pp. 643–668.
[45] C.A. Dinarello, Interleukin-1, Cytokine Growth Factor Rev. 8 (1997) 253–265.
[46] B. Magnadóttir, S. Lange, S. Gudmundsdottir, J. Bøgwald, R. Dalmo, Ontogeny of humoral immune parameters in fish, Fish Shellfish Immunol. 19 (2005) 429–439.